JOURNAL OF MAGNETIC RESONANCE, Series B **106**, 76–79 (1995)

Water Suppression in ^{1}H MASS NMR Spectra of Lipids and Biological Membranes

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Received July 6, 1994

In the study of unsonicated biological membranes or lipid suspensions, magic-angle sample spinning (MASS) is the only method that can provide high-resolution ^{1}H and ^{13}C NMR spectra (*1–4*). However, with unlabeled biological membranes, the low natural abundance of ^{13}C leads to a serious sensitivity problem (*5*). On the other hand, ^{1}H NMR provides spectra in a short time, but the strong ^{1}H water resonance (Figs. 1a, 2a, and 3a) causes many severe problems, namely excessive dynamic range, limited analog-to-digital resolution, baseline distortion, loss of resolution around the water peak, and other related difficulties (*6*).

The drastic way to suppress the water peak is to replace H_2O with D_2O (*1–4*), but, although it is possible with liposomes or lipid dispersions, in many cases it is inconvenient, and in some cases it is harmful to the cell membrane structure if it requires lyophilization or for the cell metabolism if it requires long centrifugations. Moreover, a residual HDO peak is still present and most of the amide peaks disappear due to slow exchange between H and D nuclei.

Here, we show, for the first time to our knowledge, that it is possible to apply to solid-state NMR the classical water-suppression methods used in liquid-state NMR. The first class of pulse sequences tested here requires discriminating the water peak from the others by its T_1 [the WEFT (*7*) and DASWEFT (*8*) pulse sequences] or T_2 [the WATR pulse sequence (*9*)] relaxation time. The second class of sequences involves selective excitation and selective nonexcitation of different parts of the spectrum. The jump-and-return sequence (*10*) is a very popular selective-excitation sequence which creates a sinusoidal excitation profile with a sharp null at the water frequency. For the DANTE-Z sequence (*11*), two subsequent FIDs are acquired with opposite phases around the carrier frequency and with the same phase elsewhere. By subtracting them, the magnetization adds constructively around the carrier frequency and destructively elsewhere, generating a Gaussian excitation profile. The NON-DANTE-Z sequence is an adapted version of the DANTE-Z sequence in which the carrier frequency is centered on the water peak and the two subsequent FIDs are added instead of subtracted, destroying the water magnetization and leaving the other peaks untouched. The third approach used here is classical water-peak saturation, by continuous RF irradiation except during detection (*6, 12*).

Test samples used here were egg-yolk phosphatidylcholine (egg PC) dispersions, whole erythrocytes, erythrocyte ghosts prepared as described elsewhere (*13*), and bovine heart mitoplasts. Mitoplasts were prepared by freeze–thawing mitochondria, as described in (*14*), and centrifuging them four times at 4000*g* for 20 minutes in a 125 m*M* NaCl, 10 m*M* phosphate, pH 7.6 buffer, to remove the sucrose from the sample. After preparation, the mitoplasts were used within 7 hours.

Partial water suppression was obtained with the DASWEFT pulse sequence, combining the WEFT sequence with a DANTE pulse train (Fig. 1b), but it was impossible to leave the lipid resonances untouched while suppressing the water peak with WEFT or WATR sequences alone, probably because lipids and water have relatively close T_1 and T_2 relaxation times.

Good water suppression was obtained with the jump-and-return pulse sequence (Fig. 1c), but selective excitation proved to be inconvenient for lipid studies since too large a bandwidth had to be selected, causing errors in relative intensities of the different peaks. Selective nonexcitation with the NON-DANTE-Z sequence gave better results, but only if the water peak was not too large (Fig. 1d).

Best results have been obtained with water-peak saturation (Fig. 1e), and the residual water peak is comparable to the HDO peak in a D_2O sample (Fig. 1f). With cell samples (Figs. 2a and 3a), the huge water peak overlaps membrane peaks above 3.6 ppm and creates artifactual peaks around it. After presaturation, the water peak is diminished by factors of 3 to 300, depending on the amount of water in the sample, the resolution is increased around it, and the artifactual peaks have disappeared (Fig. 2b). Reducing the dynamic range allows one to increase the receiver gain by a factor of about 30 (Fig. 3b). In consequence, we believe that relative intensities of the membrane peaks are more reliable in Fig. 3b

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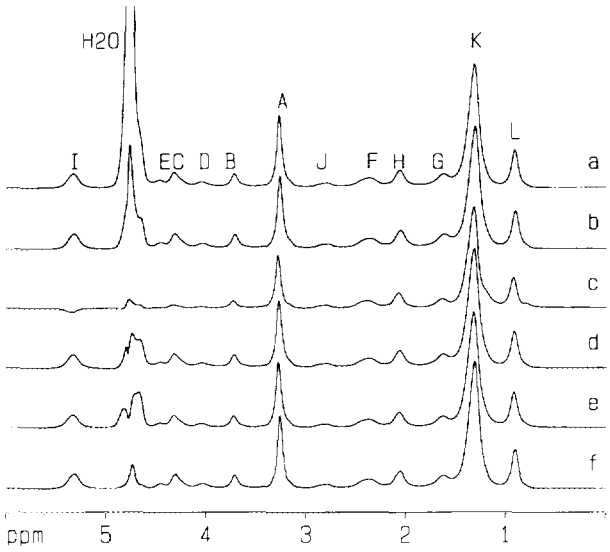

FIG. 1. ^{1}H MASS (400 MHz) spectra of 70 mg egg PC in 50% water, at 30°C, in a 4 mm rotor spinning at 4 kHz. Experimental details are as follow: ^{1}H 90° pulse of 7 μs, 3 s recycle time, eight acquisitions with classical phase cycling, and 3 Hz line broadening. (a) Normal spectrum with 90° observation pulse. (b) DASWEFT spectrum with a recovery delay of 1.2 s and 64 DANTE medium-power pulses of 2.7 μs, spaced by 50 μs. (c) Jump-and-return spectrum with a waiting time of 220 μs. (d) NON-DANTE-Z spectrum with 64 DANTE medium-power pulses of 2.2 μs, spaced by 35 μs. (e) Water-presaturation spectrum with continuous low-power irradiation at water frequency during recycle time. (f) Same as (a) but H_2O replaced by D_2O (99.99%). Proton attributions (*19*) are as follows: headgroups, A = $N(CH_3)_3$, B = NCH_2, C = $POCH_2$; glycerol, D = 3-CH_2O, E = 1-CH_2O; chain, F = CH_2CO; G = CH_2C–CO, H = CH_2–C$\equiv$C, I = CH$=$CH, J = C$\equiv$C–CH_2–C$\equiv$C, K = other CH_2, L = terminal CH_3.

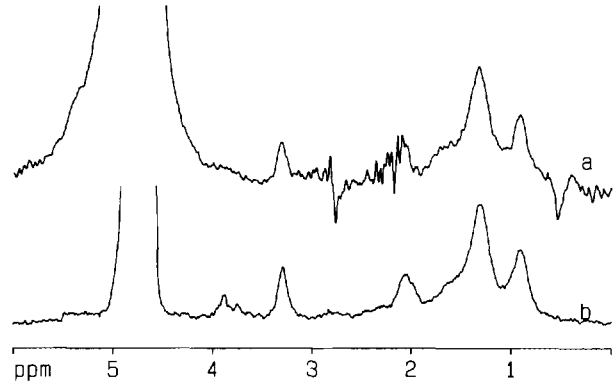

FIG. 2. ^{1}H MASS (400 MHz) spectra of 90 mg packed bovine heart mitoplasts, at 37°C, in a 4 mm rotor spinning at 4 kHz. Spectral conditions basically as in Fig. 1 but with 5 Hz line broadening. (a) Normal spectrum with 90° observation pulse. (b) Water-presaturation spectrum with continuous low-power irradiation at water frequency during recycle time.

than in Fig. 3a. It was then possible to obtain a 2D NOESY spectrum of only 90 mg of mitoplasts, under continuous water saturation, in a couple of hours (Fig. 4). Compared to a regular NOESY spectrum obtained in the same amount of time (data not shown), the resolution around the water peak and the signal-to-noise ratio were significantly increased and many artifactural F_1 lines were removed.

Presaturation seems to be a powerful technique for gaining sensitivity in ^{1}H spectra of biological membranes in water. Nevertheless, when irradiating the lipid dipersion sample at the water frequency with different presaturation pulse strengths, the signal-to-noise ratio of each lipid peak is also reduced by a small factor. Although each lipid peak seems to be divided by the same factor, at least when 0.5 ppm away from the water frequency, one should worry about probable spin diffusion within the sample and the influence of presaturation upon magnetization pathways: when two protons are cross relaxed, presaturating the first proton should affect the second proton as well (*15*). Earlier proton relaxation studies of lecithins gave rise to some controversy about the relative importance of spin diffusion for the relaxation of methylene protons (*16*). Later, the use of ^{1}H MASS NMR resolved this question when Forbes *et al.* (*3*) demonstrated the existence of spin diffusion along each phosphatidylcholine lipid, from the chain terminal CH_3 to the headgroup $N(CH_3)_3$, in unsonicated bilayers. However, this process was considered to be weak since individual T_1 relaxation times for different protons could be measured and were found to be different, although close to each other (*4*).

Proton T_1 determination using the inversion-recovery pulse sequence with continuous saturation is another way to check the presence of spin diffusion (*15, 17*). We have compared the intensities and the longitudinal relaxation times of egg PC proton peaks in D_2O or H_2O, with or without water presaturation. The relaxation-time measurements are summarized in Table 1. Relative peak intensities in 1D spec-

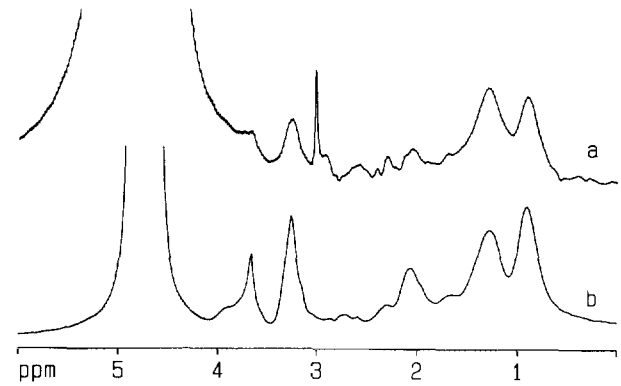

FIG. 3. ^{1}H MASS (400 MHz) spectra of 130 mg packed white erythrocyte ghosts, at 30°C, in a 4 mm rotor spinning at 4 kHz. Spectral conditions basically as for Fig. 1 but with 256 acquisitions and 5 Hz line broadening. (a) Normal spectrum with 90° observation pulse and receiver gain of 4. (b) Water-presaturation spectrum with continuous low-power irradiation at water frequency during recycle time and receiver gain of 128.

tra and NOESY cross- and diagonal-peak volumes were found to be identical under all conditions. T_1 values for lipids are a little bit higher in D_2O than in H_2O, probably due to the intermolecular dipole–dipole interaction between water and lipid spins and the lower ^2H magnetogyric ratio. Nevertheless, in H_2O, T_1 of lipid protons is far below T_1 of water protons, proving the persistence of a magnetization gradient and thus a weak spin diffusion process between water and lipids, probably because few H_2O molecules are in contact with the lipids. In any case, for most proton T_1, no significant difference is observed under water saturation and the lipid magnetization pathways seem to be largely unperturbated. The only part of the lipid that is slightly affected by presaturation is the glycerol region (1-CH_2O and 3-CH_2O), which is thought to be very close to strongly coupled water molecules.

Although it has been suggested that, in the case of a solid sample, an accurate synchronization of the rotor rotation with the RF pulses had to be performed (*18*), no influence from the absence of rotor synchronization was noticed in our lipid samples. All pulse sequences were tested on Bruker double-bearing MASS probeheads for 4 or 7 mm sample rotors, at spinning rates of 1 to 8 kHz.

In summary, some techniques of water suppression were adapted to ^1H MASS NMR. Presaturation appeared to be the most efficient method, with an increase in resolution as well as in the lipid-to-water signal ratio allowing an increase of the receiver gain by a factor of about 30. The influence of fast sample rotation upon such sequences and the influence of such sequences upon magnetization pathways were tested. Further experiments might combine these techniques with any other pulse sequences involving the ^1H nucleus for lipid studies in biomembranes or for other liquid-crystal investigations.

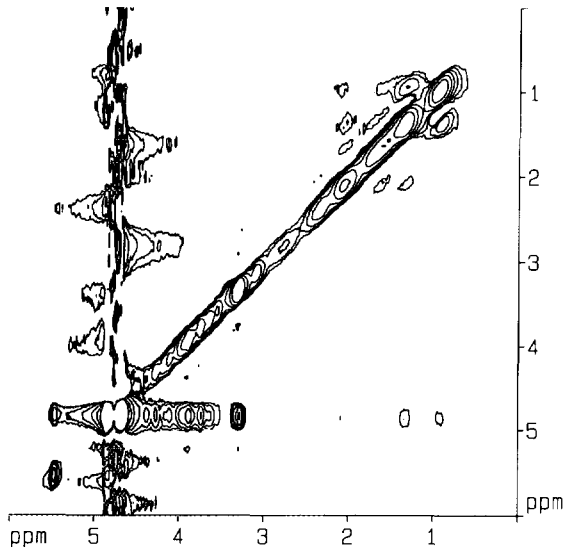

FIG. 4. Water-presaturated ^1H NOESY (400 MHz) spectrum of 90 mg packed bovine heart mitoplasts, at 37°C, in a 4 mm rotor spinning at 4 kHz. Experimental details are as follows: ^1H 90° pulse of 11 μs, continuous low-power irradiation at water frequency during 1 s recycle time and 100 ms mix time, 128 files of 2K points, 64 scans, and 4 dummy scans per file with classical phase cycling; total recording time, about 3 hours. Prior to 2D FT, the data were multiplied with a sine-bell window function shifted by 45° along each axis and zero-filled to 256 and 4K data points along the f_1 and f_2 dimensions, respectively. The spectrum baseline was then automatically corrected by subtraction of a 5-degree polynomial.

TABLE 1
Proton T_1 (s) of Egg PC at 400 MHz under Different Conditions

Proton peaks	D_2O	H_2O, no presaturation	H_2O, presaturation
H_2O		1.75	
$N(CH_3)_3$	0.43	0.42	0.41
NCH_2	0.44	0.43	0.41
$POCH_2$	0.51	0.50	0.45
3-CH_2O	0.53	0.51	0.44
1-CH_2O	0.58	0.59	0.47
CH_2CO	0.52	0.51	0.48
CH_2C–CO	0.51	0.51	0.48
CH_2–C═C	0.58	0.58	0.56
CH═CH	0.74	0.72	0.71
C═C–CH_2–C═C	0.60	0.57	0.58
Other CH_2	0.67	0.67	0.65
Terminal CH_3	1.13	1.15	1.10

Note. Error is between 1 and 5%, based on repeated measurements.

ACKNOWLEDGMENTS

The authors acknowledge the constant assistance of P. Fellmann and the help of A. Maftah and A. Zachowski in the bovine heart mitoplast sample preparation. D.E.W. thanks D. Lévy and M. Seigneuret for helpful discussions about the DANTE-Z sequence and advice on spectrometer operation. D.E.W. was a recipient of a fellowship from the Ministère de l'Enseignement Supérieur et de la Recherche. This work was supported by grants from the Université Denis Diderot, the Centre National de la Recherche Scientifique (URA 526), the Institut National de la Santé et de la Recherche Médicale, and the Association pour la Recherche contre le Cancer.

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